respectively (0.25 mg/L for total *S. aureus* and enterococci, MSSA, MRSA, VRS, and VRE). Resistance patterns noted were: tetracycline (see Table), ESBL- and fluoroquinolone resistance in Enterobacteriaceae (28.8, 33.7%, respectively), VRE (9.9%), MRSA (47.7%) and *Acinetobacter* spp. carbapenem (imipenem)-resistant (76.1%).

	Cum. % inhibited at tigecycline MIC (mg/L):							Tig	Tet
Organism (no. tested)	≤0.06	0.12	0.25	0.5	1	2	4	% S[a]	% R[b]
S. aureus (658)	10	46	99	>99	100			99.9	6.7
CoNS (221)	8	35	89	>99	100			99.5	7.7
Enterococci (292)	18	49	97	100				96.6	61.0
E. coli (291)	4	35	90	>99	>99	>99	100	99.7	47.8
Enterobacter (107)	0	4	41	81	98	100		100	16.8
Klebsiella (202)	0	3	43	85	95	99	100	98.5	29.2
Acinetobacter (205)	2	5	28	60	89	99	100	98.5	28.3

a. Tigecycline susceptibility by US-FDA and Jones et al. (2007) criteria.
b. Tetracycline resistance by CLSI criteria.

Conclusion: MDR rates across all GP and GN species have increased in Latin America. However, tigecycline remained very active against these MDR strains. Tigecycline exhibited promising spectrum/potency exceeding currently available agents against sampled isolates from Latin America.

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74.007

Genetic diversity of enterococci harboring high-level gentamicin resistance genes *aac(6′)-Ieaph(2″)-Ia* or *aph(2″)-Ie* in a Japanese hospital

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Background: Enterococci are important human pathogens implicated in various nosocomial infections. In Japan, prevalence of high-level gentamicin resistance (HLGR) is recognized as a potential concern for enterococcal infections, although vancomicin resistance is rarely found. In the present study, prevalence of two aminoglycoside-modifying enzyme (AME) genes *aac(6′)-Ie-aph(2″)-Ia* and *aph(2″)-Ie* which confer HLGR to enterococci and clonal diversity of the enterococcal isolates with these resistance genes were analyzed.

Methods: A total of 1128 clinical isolates of enterococci obtained in a Japanese hospital during a period between 1997 and 2007 were analyzed for presence of *aac(6′)-Ie-aph(2″)-Ia* and *aph(2″)-Ie* by PCR. IS*256*-flanking patterns, sequence diversity of these genes were analyzed by PCR and direct sequencing. *Enterococcus faecalis* and *Enterococcus faecium* stains with the HLGR genes were typed by multi-locus sequence typing (MLST).

rococcal species, respectively, and *aph(2″)-Ie* was detected in 3.3% of *E. faecium* isolates. Two IS*256*-flanking patterns, truncated forms of Tn*5281* lacking IS*256* at only 5′-end and both 5′ and 3′ ends of *aac(6′)-Ie-aph(2″)-Ia* were the most prevalent. Among 14 *E. faecalis* and 10 *E. faecium* strains harboring *aac(6′)-Ie-aph(2″)-Ia*, eight and six different sequence types (STs) were identified by MLST, respectively. STs of most of the *E. faecium* strains belonged to the clonal complex CC17 which is known as globally emerging lineage of vancomycin- or ampicillin-resistant *E. faecium* clones. In contrast, *E. faecium* strains with *aph(2″)-Ie* were classified into newly assigned STs (ST426 or its single locus variant ST427).

Conclusion: HLGR gene *aac(6′)-Ie-aph(2″)-Ia* was distributed to *E. faecalis* with various genetic lineages and *E. faecium* with lineages in CC17 mostly. The *aph(2″)-Ie* was carried by *E. faecium* from a few limited lineages.

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74.008

The expansion of ST80-SCC*mec*-IV clone of community-acquired methicillin resistant *Staphylococcus aureus* in Kuwait hospitals

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Background: Community- acquired methicillin resistant *S. aureus* (CA-MRSA) that infects patients with no traditional risk factors for the acquisition of MRSA infections is increasing in many parts of the world. In this study, CA-MRSA obtained from patients in eight Kuwait hospitals were characterized for their antibiotic resistance and typed using pulsed-field gel electrophoresis (PFGE), SCC*mec* and multilocus sequence typing (MLST) to ascertain their relatedness.

Methods: In total 135 CA-MRSA isolates were obtained from eight hospitals in Kuwait between 1 January 2005 and 31 December 2006. Antibiotic susceptibility testing was performed by the disk diffusion method. MIC was determined using Etest strips. PFGE was performed utilizing *Sma*I digestion of genomic DNA followed by fragment separation in

a CHEF- DRIII system. SCC*mec* typing and MLST were performed according to internationally standardized protocols.

Results: They were resistant to kanamycin (62%), fusidic acid (42%), tetracycline (39.3%), erythromycin and clindamycin (21.5%), gentamicin (5.9%), streptomycin (6.7%), trimethoprim (5.9%), mupirocin (6.6%) cadmium acetate (82.2%) and ethidium bromide (12.6%). All were susceptible to vancomycin, teicoplanin and linezolid. One hundred and three (76.3%), 11 (8.14%), 9 (6.67%) and 12 (8.9%) isolates carried SCC*mec* type IV, SCC*mec* —Iva, SCC*mec*-IVc and SCC*mec* - V genetic elements respectively. PFGE yielded 10 PFGE types and subtypes with the majority of them belonging to PFGE type 1 and subtypes (47.4%), type 2 (22.2%), type 3 (3.7%), type 4 (14.0%). Other PFGE types were present in small numbers MLST revealed 10 sequence types comprising ST80 (46.6%), ST30 (10.7%), ST5 (19.3%), ST6 (10.7%), ST8 (3.6%), ST46 (3.6%), ST88 (3.6%), ST834 (3.6%) and ST950 (3.6%). Isolates belonging to the same PFGE pattern had the same sequence type.

Conclusion: Although the isolates belonged to 10 different sequence types, the ST80-SCC*mec* IV clone belonging to PFGE type 1 and subtypes was the most prevalent clone. Its presence in all eight hospitals shows its continuing expansion in Kuwait hospitals.

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A novel multiplex real-time PCR assay for CA-MRSA: Rapid typing of SCCmec type assignment with detection of the pathogenicity

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Background: Numerous community-associated MRSA (CA-MRSA) infections have been seen in healthy populations. To detect CA-MRSA is important for clinicians because many fatal cases were reported. Staphylococcal cassette chromosome mec (SCCmec) typing is useful for defining CA-MRSA clones. The rapid detection system for CA-MRSA is needed. We established a convenient multiplex real-time PCR for detection of SCCmec type assignment with detection of the pathogenicity analyzed MRSA clones in Nagasaki.

Methods: 776 MRSA isolated from different clinical specimens, sputum, pus and blood at Nagasaki University Hospital from Jan. 2000 to Dec. 2007. All isolates were subjected to MIC testing and PCR for *TSST-1*(toxic shock syndrome toxin 1), *sec* (enterotoxin type c), *etb*(exfoliative toxin type b), and *PVL*(Panton-Valentine Leucocidin). PCR was performed using a LightCycler 480 to amplify a total of 10 genes in the same run. The entire run time for this assay is approximately 4 hours. Based on these molecular typing methods, we characterized the genetic background of MRSA strains isolated in our hospital. The medical records were also reviewed for the determination of nosocomial infection or the community acquired infection.

Results: The 667 MRSA clones detected from pus were classified as SCCmec type II (77.7%), SCCmec type IV (19.2□), and SCCmec type I (3.0□). SCCmec type IV clones has been increasing for 8years. 87.5% of SCCmec type II clone had *TSST-1* and *sec* genes. 15 isolates were *etb* positive, all of them isolated from pus. No isolate was *PVL* positive. Most patients infected SCCmec type IV clone were classified as nosocomial infection.

Conclusion: Our system was thus the convenient and reliable method for typing MRSA in Japan. SCCmec type II MRSA which possesses *TSST-1* and *sec* genes was the major nosocomial infection type in Japan. The present study indicated the high rates of PVL negative SCCmec type IV in Nagasaki, and reveals for the drift of MRSA clones mixed the type of nosocomial infection and the type of community acquired infection.

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74.010

Trend of vancomycin MIC values among MRSA clinical isolates and association with patient outcome

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a common cause of nosocomial and community-acquired infection. Vancomycin has become the drug of choice given the emergence of MRSA; however, studies have reported an increase in the vancomycin minimum inhibitory concentration (MIC) and vancomycin treatment failure despite MICs within the susceptible range ()2 μg/mL). Limited studies have examined whether this increase in vancomycin MIC is associated with patient outcome.

Methods: We reviewed the medical records of patients diagnosed with MRSA bloodstream or lower extremity wound infection from January 1, 1998 through December 31, 2008. Bivariate and multivariate analyses were conducted to examine the association between vancomycin MIC and other covariates.

Results: 97 patients were diagnosed with a MRSA infection; 65% (63/97) of patients had a bloodstream infection and 35% (34/97) of patients had a wound infection. From 1998 to 2003, MRSA with a low vancomycin MIC ($\leq$1 μg/mL) were in the majority; however, from 2004 to 2008, MRSA with a high MIC (=2 μgg/mL) were in the majority. Therefore, over time, there was a significant upward trend in vancomycin MIC values ($p = 0.01$). Logistic regression analysis revealed that a high vancomycin MIC was significantly associated with a past medical history of malignancy ($p = 0.04$) and death within 30 days of infection ($p = 0.04$) compared to a low vancomycin MIC.

Conclusion: Our study has shown (1) vancomycin MIC values have displayed an upward 'creep' over time, and (2) high MIC values of vancomycin is significantly related to a past medical history of malignancy as well as higher mortality within 30 days of MRSA infection. Further prospective studies are needed to examine the clinical significance of an upward 'creep' in vancomycin MIC values.

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